Kong et al., http://www.jcb.org/cgi/content/full/jcb.201407087/DC1

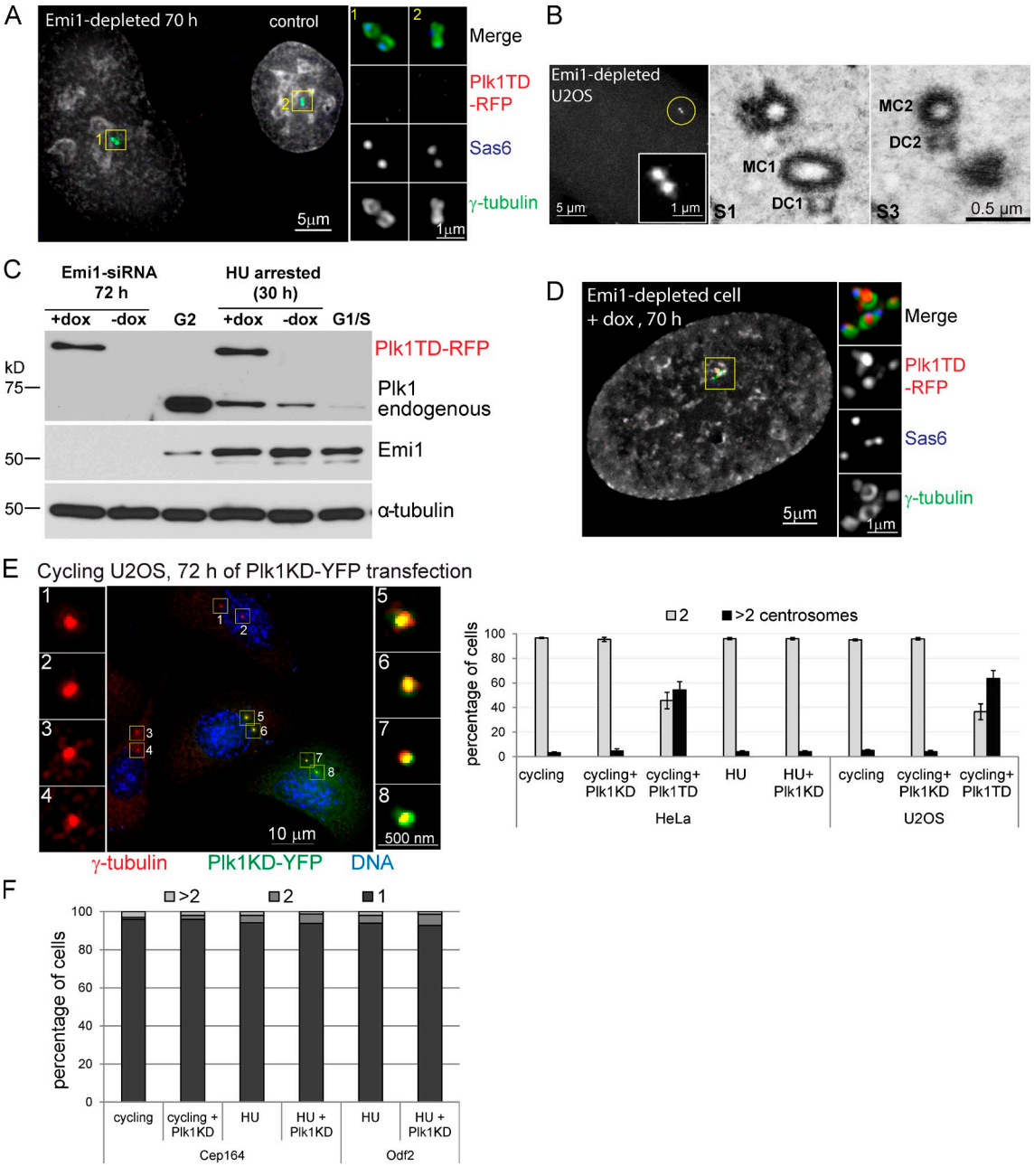


Figure S1. **Expression of active Plk1 promotes centriole reduplication in endocycling Emi1-d cells.** (A) Emi1-d cells have two γ-tubulin signals, each associated with one Sas6 signal, indicative of two duplicated centrioles. Plk1TD-RFP is not induced. (B) CLEM analysis of centrioles in Emi1-d cells. Two C1-GFP signals are visible by fluorescent microscopy. EM analysis of the same centrioles reveals two MCs each associated with a ∼100-nm-long DC. An enlarged C1-GFP signal (encircled in yellow) is shown in the inset. (C) Endogenous Plk1 is undetectable in Emi1-d cells by Western blotting. The cells express physiological levels of T210D-RFP upon induction by doxycycline (dox). (D) Induction of Plk1TD-RFP in Emi1-d cells induces disengagement and subsequent centriole reduplication, judging by the increased number of γ-tubulin and Sas6 signals. (E and F) Expression of kinase-dead mutant of Plk1 (Plk1KD) in cycling or HU-arrested cells does not promote centriole disengagement, reduplication, or accumulation of appendage proteins on the centrioles. Plk1KD was transfected into cycling or HU-arrested cells. Cycling cells were fixed for 72 h, HU-arrested 36 h later, and labeled for γ-tubulin, Cep164, or Odf2. (E) Quantification of γ-tubulin signals (n = 300 for each condition for three experiments). The error bars represent the means and SD. (F) Quantification of Cep164 or Odf2 signals (n = 100). Associated with Fig. 1 and Fig. 3.

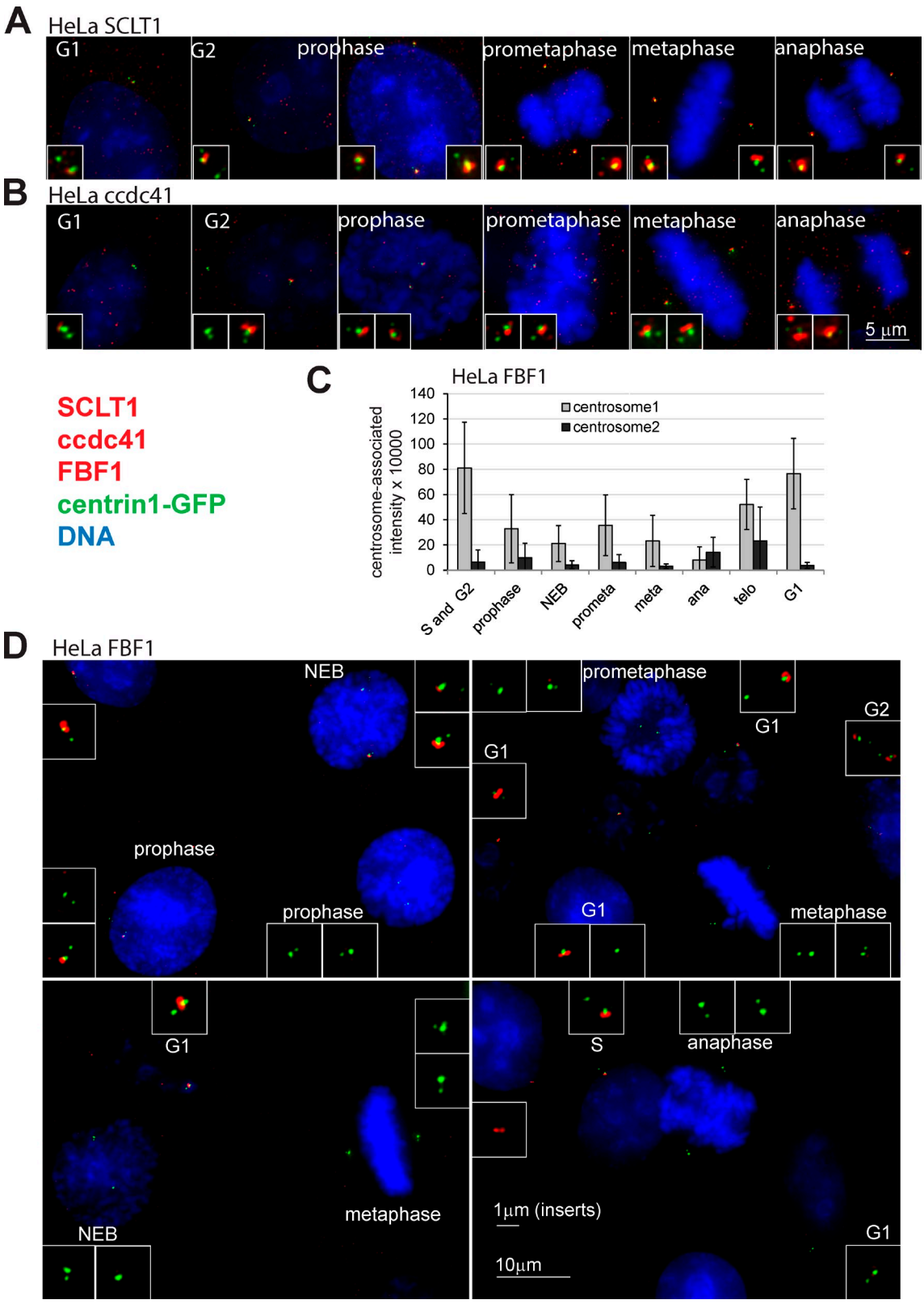
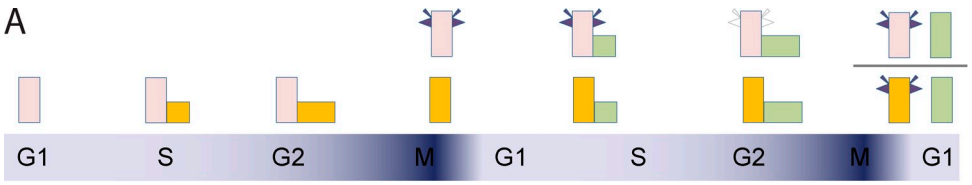
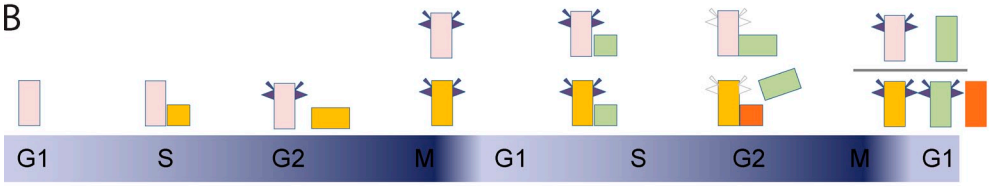


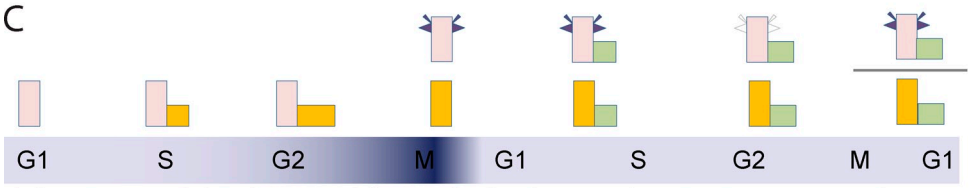
Figure S2. **Appendage protein FBF1 is diminished or undetectable on the centrosomes of mitotic HeLa cells.** Cycling cells were fixed and stained for appendage proteins SCLT1, ccdc41, and FBF1. (A and B) SCLT and ccdc41 associate with one MC during interphase but with both MCs during mitosis. Intensities of the signals were higher in mitosis. (C) Quantification of centrosome-associated FBF1. The error bars represent the means and SD. (D) Examples of interphase and mitotic to illustrate the absence of a FBF1 signal on MCs in mitosis. Associated with Fig. 4. Enlarged centrosomes are shown in the insets. meta, metaphase; NEB, nuclear envelope breakdown; ana-telo, anaphase-telophase.



Unperturbed two consecutive cell cycles with controlled, timely regulated Plk1 activity. Plk1 activity is low during G1 and S and peaks during G2 and the first part of M. Nascent centrioles gradually mature through two cell cycles.



Cells expressing near physiological level of active Plk1 mutant. Plk1 activity is low during G1 due to Plk1 degradation but is present on the centrosomes from early S to M. The centriole maturation process accelerates.



If Plk1 activity is inhibited in centriole's second cell cycle, centriole maturation is not complete and appendage assembly on young mother centrioles is compromised. Nascent centrioles cannot reach full length.

Hydroxyurea treatment or Emi1-depletion. In cells arrested in interphase with low/ without Plk1 activity, maturation of young centrioles does not occur.

Hydroxyurea treatment or Emi1-depletion with induction of active Plk1. Cells arrested in interphase with high Plk1 activity. Centriole maturation and reduplication occurs within one cell cycle in the absence of mitotic progression.

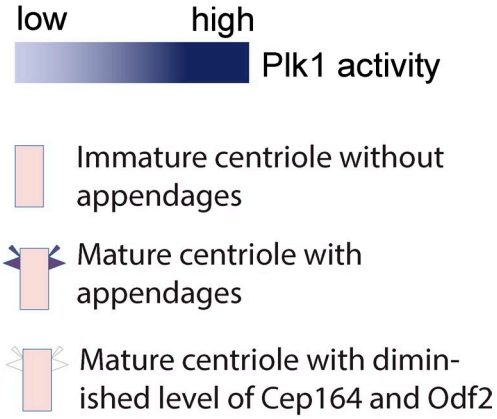
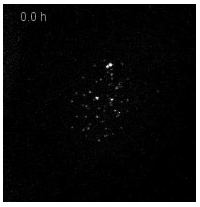


Figure S3. **Controlled Plk1 activity through two consecutive cell cycles is necessary for timely centriole maturation.** Scheme summarizes how dysregulation of Plk1 activity influences centriole cycle.

Table S1. **Immunofluorescence analysis of the centrosomes in Emi1-d cells before and after Plk1TD-RFP expression**

Protein	Emi1-d cells							Comment
	No Plk1TD		Plk1TD 46 h		Plk1TD 70 h			
	MC	DC	MC	DC1	MC	DC1	DC2	
Plk1TD-RFP	–	–	+	+	+	+	+	Associates with DCs during disengagement and onward, and intensity on DCs increases with time
hSas6	–	+	–	±	–	–	±	Gradually disappears from DCs after disengagement; associated with all nascent centrioles
Centrobin	±	+	±	+	±	±	+	Gradually disappears from DCs after disengagement; associated with all nascent centrioles
Cep152	+	–	+	–	+	+	–	Full or partial rings gradually form around DCs after their disengagement; please see Fig. 1.
CPAP	+	+	+	+	+	+	+	Associates with DCs during disengagement and onward
Cep135	+	±	+	+	+	+	+	Associates with DCs during disengagement and onward
Cep120	+	+	+	+	+	+	+	Associates with DCs during disengagement and onward
γ-Tubulin	+	–	+	+	+	+	+	Associates with DCs during disengagement and onward, and intensity on DCs increases with time.
Cep170	+[a]	–	+[b]	+	+[b]	±	±	Accumulates early after disengagement on most DCs
Cep164	+[a]	–	+[b]	±	+[b]	±	±	Accumulates on some DCs after disengagement; please see Fig. 1.
Odf2	+[a]	–	+[b]	–	+[b]	±	±	Accumulates on some DCs after disengagement; please see Fig. 1.
Centrin1-GFP	+	+	+	+	+	+	+	Associates with all centrioles from their formation
hPOC5	+	–	+	+	+	+	+	Associates with DCs during disengagement and onward
GT335	+	–	+	+	+	+	+	Associates with DCs during disengagement and onward

Plk1TD, constitutively active Plk1 T210D mutant; MC, original mother centriole; DC, engaged daughter centriole; DC1, first generation of disengaged DCs; DC2, second and subsequent generations of disengaged DCs; CPAP, centrosomal P4.1-associated protein; +, associated with a centriole; –, undetectable; ±, sometimes associated with the centriole. Associated with Fig. 1.
[a]Associated with only one MC.
[b]Associated with both MCs.



Video 1. **Localization and dynamics of Plk1TD-RFP in cycling U2OS cells.** Expression of Plk1TD-RFP was induced in U2OS cells by dox. 20 h later, cells growing on the coverslip were assembled into the Rose chamber and imaged by an inverted microscope (Eclipse Ti; Nikon) equipped with spinning-disk confocal microscope (Yokogawa Electric Corporation), a back-illuminated 16-µm-pixel electron-multiplying charge-coupled device camera (DU897; Andor Technology), and a 60×, NA 1.45 Plan Apochromat TIRF objective. 200-nm z sections spanning the entire volume of interphase cells were taken every 5 min. Maximal intensity projections are shown. The almost physiological level Plk1TD-RFP in a U2OS cell is undergoing a typical cell cycle–dependent regulation and relocalization to different cellular structures, such as kinetochores, centrosomes, and the midbody.

An Excel file is also included that shows a distance calculator for multichannel 3D images used in this study.